Straightforward Synthesis of *N*-Hydroxy Peptides

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Received 30 January 2003

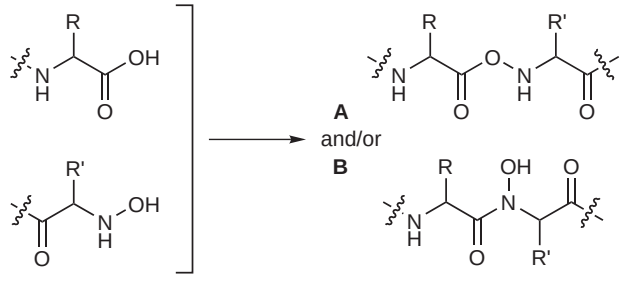
Abstract: *N*-Hydroxy dipeptides are readily synthesized by reduction of the corresponding oximes. The two diastereomers obtained are easily separated by flash chromatography. They can be coupled with a third amino acid moiety – without protection of the hydroxyl group – to give *N*-hydroxy tripeptides.

Key words: *N*-hydroxy peptides, oximes, reduction, hydroxylamines, N-/O-acylation

There has been a growing interest in *N*-hydroxy peptide type compounds[1] in the last decade. This modified peptidic structure – including one or more hydroxamate motifs – has been observed in some natural products such as the apoptosis inducers polyoxypeptins A and B.[2] The anti-HIV activity of some other *N*-hydroxy pseudopeptides has been tested.[3] Moreover the additional OH group induces conformational changes in the peptide.[4] Furthermore the strong iron complexation properties of the hydroxamate moiety was used for the design of good siderophores.[5]

The obvious method for the synthesis of these compounds would be the direct coupling of a *N*-hydroxy amino acid (or a terminal *N*-hydroxy peptide, Scheme 1) with an activated peptide residue. However, two coupling products can be obtained: the *O*-acylated compound **A** and the desired *N*-acylated compound **B**. Protection of the OH group as a benzyl ether has thus been used to overcome this problem.[6] This requires an additional deprotection step but above all *N*-benzyloxy amino acids show a decreased reactivity in coupling reactions when bulky residues are at stake.[7]

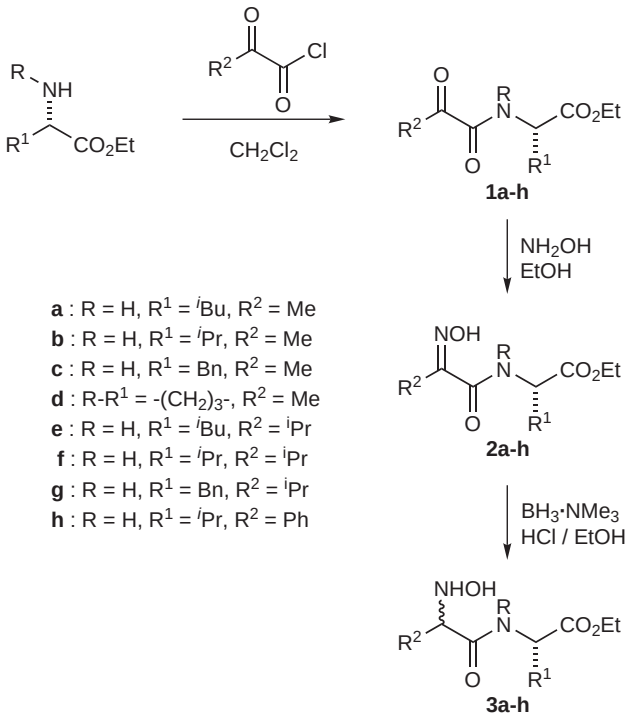
In this communication we report (i) the reduction of peptidic oximes as a way to terminal *N*-hydroxy dipeptides and (ii) two methods for obtaining inner *N*-hydroxy peptides – involving or not a transient protection of the hydroxyl group during the coupling with another peptidic fragment.



Scheme 1

Routes to non-racemic *N*-hydroxy α-amino acids are known[8] but require multi-step procedures from optically active starting materials. We preferred to investigate the reduction of peptidic oximes **2** as a direct synthesis of terminal *N*-hydroxy dipeptides **3**, in order to have rapid access to a small library of molecules. Compounds **2** were obtained in two steps from the corresponding α-amino esters via the α-keto amides **1** (Scheme 2). The results are summarized in Table 1.

Acid chlorides were generated by reaction of the corresponding α-keto acids with α,α-dichloromethylmethylether[9] and were either isolated (Entries 1–4 and 8) or prepared in situ (Entries 5–7). They were then reacted with various α-amino esters – HLeuOEt (Entries 1, 5), HValOEt (Entries 2, 6, 8), HPheOEt (Entries 3, 7) and HProOEt (Entry 4) – to give α-keto amides **1** in good yields. Condensation of **1** with hydroxylamine hydrochloride in ethanol followed by addition of triethylamine led to oxime **2** as a single isomer (R^2 = Me) or as a mixture of *E* and *Z*-isomers (R^2 = *i*-Pr, Ph).[10] Reduction of **2** was car-



a : R = H, R^1 = ^{i}Bu, R^2 = Me
b : R = H, R^1 = ^{i}Pr, R^2 = Me
c : R = H, R^1 = Bn, R^2 = Me
d : R-R^1 = -$(CH_2)_3$-, R^2 = Me
e : R = H, R^1 = ^{i}Bu, R^2 = ^{i}Pr
f : R = H, R^1 = ^{i}Pr, R^2 = ^{i}Pr
g : R = H, R^1 = Bn, R^2 = ^{i}Pr
h : R = H, R^1 = ^{i}Pr, R^2 = Ph

Scheme 2

Synlett 2003, No. 5, Print: 10 04 2003.
Art Id.1437-2096,E;2003,0,05,0671,0674,ftx,en;D02503ST.pdf.

ISSN 0936-5214

Table 1 Synthesis of Compounds **1**, **2** and **3**[13]

Entry	Compound	Yield (%)		
		1[a]	**2**[a]	**3**[b]
1	**a**	87	86	80
2	**b**	80	90	93
3	**c**	86	81	80
4	**d**	93	95	40
5	**e**	65[c]	86	88
6	**f**	76[c]	70	91
7	**g**	55[c]	73	83
8	**h**	95	84	no reaction

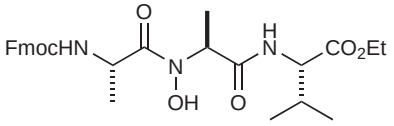
[a] Isolated yields.
[b] Total yield of the two diastereomers.
[c] Starting from keto acid; the acyl chloride was not isolated.

ried out with 2 equivalents of trimethylamine/borane complex in 7 N ethanolic hydrochloric acid.[11] α-Oximino amides **2** bearing an alkyl R^2 substituent (Entries 1 to 7) were generally reduced in high yields whereas an aromatic R^2 substituent seems detrimental to the reactivity (Entry 8).[12]

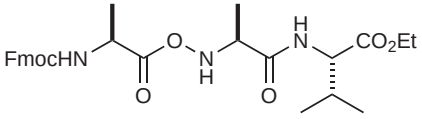
The diastereoselectivity of the reduction was poor in all cases, leading roughly to a 1:1 mixture of (*R*,*S*) and (*S*,*S*) *N*-hydroxy dipeptides **3** as estimated from the ^{1}H NMR spectra of the crude materials and/or by isolated yield of each diastereomer after separation by liquid chromatography.[14] The absolute configuration of each diastereomer was assigned by hydrogenation of the *N*-hydroxylamine in the presence of Raney nickel and comparison of the dipeptide obtained with an authentic (*S*,*S*) sample prepared using standard peptide coupling methods.

Scarce examples of coupling methods for the preparation of inner *N*-hydroxy peptides can be found in the literature.[15] In our hands, coupling between FmocAlaOH and (*S*,*S*)-**3b** led to two isomers showing different ^{1}H NMR spectra. The products resulting from *N*-acylation (**4**)[16] and *O*-acylation (**5**)[17] (Figure 1) were identified by comparison with an authentic sample of Fmoc-AlaΨ[CO(NOH)]AlaVal-OEt (prepared via the *N*-benzyloxy dipeptide). All the coupling conditions that we used (activation with DCC/HOBt, EDCI, BOP, DMTMM)[18,19] gave **5** as the major product.

To avoid this *O*-acylation we envisaged a temporary *O*-protection of the hydroxylamine as a trimethylsilyl ether[20] as shown in Scheme 3. In our tuned conditions, the *O*-protected compound was obtained in situ.[21] Addition of *N*-Fmoc-Alanine chloride followed by an aqueous acidic work-up removing the TMS protecting group gave access to the *N*-hydroxy tripeptide **4** in 70% yield after recrystallization.

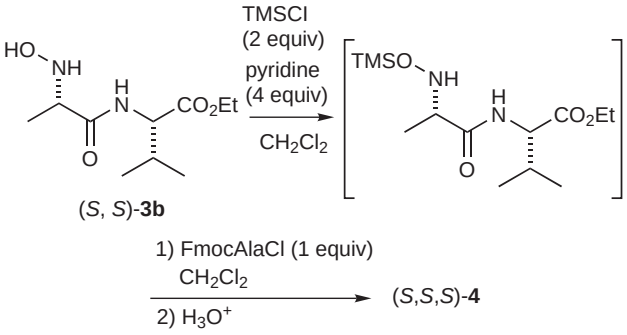


(*S*, *S*, *S*)-**4**



(*S*, *S*, *S*)-**5**

Figure 1



Scheme 3

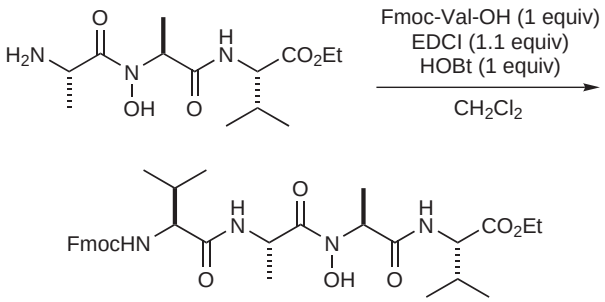
This one-pot procedure was next extended to the preparation of other *N*-hydroxy peptides (Table 2, Method A). The coupling between two Alanine residues succeeded in fair to good yields and both diastereomers of **3** show the same reactivity (compare Entries 2/3, 6/7, 8/9). However increasing the bulkiness of one of the partners gave poorer results (Entries 2/4, 7/9/10, 14). Running the reaction at 40 °C improved the yield to some extent (Entry 11).

We then reinvestigated the direct reaction with an acyl chloride. Reaction of *N*-Fmoc-Alanine chloride (1 equiv) with (*S*,*S*)-**3b** in the presence of pyridine (1 equiv) led to a 65:35 mixture of **4**/**5** in 50% isolated yield. Changing the base to sodium hydrogenocarbonate (3 equiv) resulted in a dramatic improvement, the *N*-hydroxy tripeptide **4** was produced in 82% yield. With these new conditions the coupling reactions occured in high yields, regardless of steric hindrance (Table 2, Method B). Even the reaction involving two Valine residues can be performed successfully (Entry 15).

Finally, the *N*-hydroxy tripeptide **4** was deprotected quantitatively with piperidine. Coupling with *N*-Fmoc-Valine using a standard method produced the *N*-hydroxy tetrapeptide Fmoc-ValAlaΨ[CO(NOH)]AlaVal-OEt in 76% yield (Scheme 4).

In conclusion, we have prepared a series of *N*-hydroxy peptides using a straightforward sequence. Once the inner *N*-hydroxy tripeptide is obtained, it can be further elongated via classical peptide synthesis without protection of the

hydroxyl group. We are currently broadening the scope of this methodology to incorporate other *N*-hydroxy amino acid residues with (*R*) or (*S*) configurations.



Scheme 4

Table 2 Synthesis of *N*-Hydroxy Peptides

Entry	Fmoc-AA_1-Cl	NHOH-AA_2-AA_3-OEt **3**		Method A[a]	Method B[b]
				Yield[c] (%)	Yield[c] (%)
1	Gly	AlaLeu	(*R*,*S*)-**a**	66	85
2	Ala	AlaLeu	(*S*,*S*)-**a**	69	–
3	Ala	AlaLeu	(*R*,*S*)-**a**	64	84
4	Phe	AlaLeu	(*S*,*S*)-**a**	51	76
5	Phe	AlaLeu	(*R*,*S*)-**a**	33	77
6	Ala	AlaVal	(*S*,*S*)-**b**	70	–
7	Ala	AlaVal	(*R*,*S*)-**b**	67	82
8	Phe	AlaVal	(*S*,*S*)-**b**	57	68
9	Phe	AlaVal	(*R*,*S*)-**b**	58	–
10	Val	AlaVal	(*R*,*S*)-**b**	25	78
11	Val	AlaVal	(*R*,*S*)-**b**	40[d]	–
12	Ala	AlaPhe	(*S*,*S*)-**c**	–	85
13	Ala	AlaPhe	(*R*,*S*)-**c**	63	–
14	Ala	ValVal	(*S*,*S*)-**f**	26	77
15	Val	ValVal	(*S*,*S*)-**f**	–	73
16	Gly	AlaLeu	(*R*,*S*)-**a**	66	85

[a] Method A: 1) **3**, TMSCl (2 equiv), pyridine (4 equiv), CH_2Cl_2, r.t., 30 min. 2) Fmoc-AA_1-Cl (1 equiv), CH_2Cl_2, O °C, 10 min, then r.t., 2 h. 3) H_3O^+.
[b] Method B: **3**, Fmoc-AA_1-Cl (1.03 equiv), $NaHCO_3$ (3 equiv), CH_2Cl_2, r.t., 2 h.
[c] Isolated yields. A hyphen indicates the reaction was not performed under the current method.
[d] Reaction carried out at 40 °C.

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(7) Formation of the *N*-benzyloxy amide link is very sensitive to steric hindrance and generally requires strong acylating systems such as HATU {*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate} see: (a) Akiyama, M.; Iesaki, K.; Katoh, A.; Shimizu, K. *J. Chem. Soc., Perkin Trans. 1* **1986**, 851. (b) Bianco, A.; Zabel, C.; Walden, P.; Jung, G. *J. Peptide Sci.* **1998**, *4*, 471.

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(10) The two oxime isomers are separated by liquid chromatography. No difference in diastereoselectivity was observed in their reduction so that they were used as a mixture.

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(12) Reduction of **2h** with sodium cyanoborohydride was also inefficient.

(13) All new compounds gave spectroscopic and analytical data in agreement with the assigned structures. Selected example: *N*-hydroxy dipeptide (*S*,*S*)-**3b**: $[\alpha]_D^{25}$ –3.8 (*c* 2.26, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.08 (d, ^3J = 9.6 Hz, 1 H, CONH), 5.46 (br, 2 H, N*HOH*), 4.61 (dd, ^3J = 9.6 and 4.8 Hz, H, C*H*α Val), 4.21 (m, 2 H, O$CH_2$$CH_3$), 3.66 (q, ^3J = 7.1 Hz, 1 H, C*H*α Ala), 2.23 (m, 1 H, C*H*-*i*-Pr), 1.29 (t, ^3J = 7.0 Hz, 3 H, OCH_2CH_3), 1.26 (d, ^3J = 7.1 Hz, 3 H, CH_3 Ala), 0.97 (d, ^3J = 6.9 Hz, 3 H, CH_3-*i*-Pr), 0.91 (d, ^3J = 6.9 Hz, 3 H, CH_3-*i*-Pr). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 174.0, 172.8, 62.2, 61.6, 56.7, 31.2, 19.2, 17.7, 15.6, 14.3. MS (CI):

m/*z* = 233 (100, M + H^+), 187 (60), 159 (53). IR (KBr, cm^{-1}): 3323 (m), 3254 (w), 2977 (m), 1738 (s), 1663 (s), 1541 (s).

(14) Typical example: reduction of 9.33 mmol of **2b** gave after flash chromatography (silica gel; CH_2Cl_2–MeOH, 97:3) 3.82 mmol of the first diastereomer, 0.53 mmol of a 1:1 mixture (estimated from ^1H NMR) and 3.69 mmol of the second diastereomer.

(15) See ref. 1 and references therein.

(16) Compound (*S*,*S*,*S*)-**4** {Fmoc-$Ala_1\Psi$[CO(NOH)]Ala_2Val_3-OEt}: $[\alpha]_D^{25}$ –12.7 (*c* 1.28, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 8.94 (s, 1 H, OH), 7.73 (d, ^3J = 7.4 Hz, 2 H, C*H*ar Fmoc), 7.58 (d, ^3J = 7.4 Hz, 2 H, C*H*ar Fmoc), 7.37 (dd, ^3J = 7.4 and 7.4Hz, 2 H, C*H*ar Fmoc), 7.27 (dd, ^3J = 7.4 and 7.4 Hz, 1 H, C*H*ar Fmoc), 7.27 (dd, ^3J = 7.4 and 7.4 Hz, 1 H, C*H*ar Fmoc), 7.16 (br d, ^3J = 8.7 Hz, 1 H, N*H* Val_3), 5.96 (d, ^3J = 7.0 Hz, 1 H, N*H* Ala_1), 5.29 (q, ^3J = 7.0 Hz, 1 H, C*H*α Ala_2), 4.97 (dq, ^3J = 7.0 and 6.8 Hz, 1 H, C*H*α Ala_1), 4.50 (dd, ^3J = 8.7 and 4.9 Hz, 1 H, C*H*α Val_3), 4.38–4.27 (m, 2 H, C*H*H and C*H* Fmoc), 4.23–4.16 (m, 1 H, CH*H* Fmoc), 4.17 (q, ^3J = 7.1 Hz, 2 H, OCH_2CH_3), 2.21–2.09 (m, 1 H, C*H*-*i*-Pr Val_3), 1.49 (d, ^3J = 7.0 Hz, 3 H, CH_3 Ala_2), 1.42 (d, ^3J = 6.8 Hz, 3 H, CH_3 Ala_1), 1.23 (t, ^3J = 7.1 Hz, 3 H, OCH_2CH_3), 0.91 (d, ^3J = 6.9 Hz, 3 H, CH_3-*i*-Pr Val_3), 0.88 (d, ^3J = 6.9 Hz, 3 H, CH_3-*i*-Pr Val_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.8, 172.3, 171.7, 156.4, 143.9, 141.4, 127.8, 127.2, 125.3, 120.1, 67.4, 61.6, 57.3, 54.3, 47.2, 47.2, 31.5, 19.0, 18.2, 17.8, 14.5, 14.3. MS (CI): *m*/*z* = 543 (27, M + NH_4^+) 304 (100), 179 (69). IR (KBr, cm^{-1}): 3316 (s br), 2964 (m), 1734 (s), 1701 (s), 1641 (s), 1533 (s), 1450 (s).

(17) Selected ^1H NMR data (250 MHz, $CDCl_3$) for compound **5**: δ = 7.42 (br d, ^3J = 4.5 Hz, 1 H, -NH-O-CO-), 3.73 [qd, ^3J = 6.9 and 4.5 Hz, 1 H, -C*H*(CH_3)-NH-O(CO)-].

(18) Abbreviations: DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; EDCI, 1-ethyl-3-(3′-dimethyl-aminopropyl)carbodiimide; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluoro-phosphate; DMTMM, 4-(4, 6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium chloride.

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(21) Running the reaction in $CDCl_3$ in a NMR tube with 3 equiv of pyridine indicates that with 0.7 equiv of TMSCl two exchanging products are first visible. After the addition of an excess TMSCl (up to 2.2 equiv), only one product remains. This is in agreement with previous findings (see ref. 20a).